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Inhibiting Fatty Acid Synthase in Rapidly Dividing Cells: Synthesis of 5-(alkylthio)-1H-benzo[d]imidazole-2,6-diones

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Inhibiting Fatty Acid Synthase in Rapidly Dividing Cells: Synthesis of 5-(alkylthio)-1H-benzo[d]imidazole-2,6-diones

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Abstract:

Prostate and breast cancer are the number one and number two cancer sites, respectively, for male and females within the United States.[1] Increased levels of the enzyme fatty acid synthase have been found in rapidly dividing cancer cells. This enzyme is responsible for the *de novo* synthesis of fatty acids in human cells, which are essential to cell growth and survival. The goal then for this research is to synthesize and design a new synthesis of 5-(alkylthio)-1H-benzo[d]imidazole-2,6-diones intended to successfully inhibit fatty acid synthase (FASN) by binding allosterically to the thioesterase domain of FASN. Thus by selectively inhibiting FASN in rapidly dividing cells, the production of fatty acids is inhibited and cancer cells starve. The focus of the current research is to carry out the oxidation of 5-hydroxy-1,3-dihydro-2H-benzimidazole-2-one to 1H-benzimidazole-2,6-dione through the use of sodium hypochlorite as an oxidizing agent. The product was successfully synthesized and purified. The next step is the addition of an alkylthio group to the six membered ring.

Introduction:

According to the United States Cancer Statistics (USCS) published by the Centers for Disease Control and Prevention (CDC) among the top 10 cancer sites in males and females in the United States across all races, prostate and female breast cancer come in number one and number two respectively. The statistics show that from the year 2008-2012 prostate cancer affected 131.8 people out of a 100,000, while breast cancer affected 123.1 people out of 100,000. After prostate and female breast cancer as affected areas, the numbers drop to almost half the number of people affected for lung and bronchus cancer. These trends are seen in **Figure 1**.

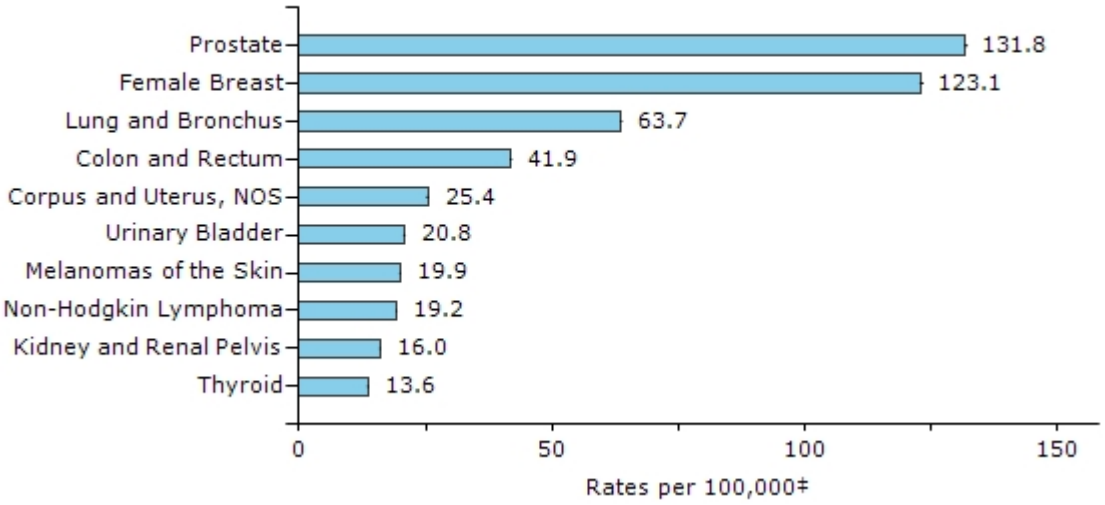

Figure 1. Graph of top 10 cancer sites for male and females within the United States from 2008-2012 for all races. (Information provided by the CDC[1].)

With prostate and breast cancer affecting more people than the following eight cancers from the list combined, we are made aware of the tremendous affect of these two cancers. Thus the goal of this research, is to target the two most prominent forms of cancer—prostate and breast cancer, through the synthesis of a possible antineoplastic agent.

Fatty acids are necessary in order to sustain the rapid cellular division that is evidenced in cancer cells. They are required for membrane regeneration, protein modification, and bioenergetics requirements.[2] The source of these fatty acids is either dietary intake or synthesis of them by the cell itself. An interesting finding that prompted this research is that once a normal cell mutates into a cancer cell, increased levels of fatty acid synthase (FASN) is found within the cell.[2] Fatty acid synthase is a multi-enzyme protein that catalyzes cellular fatty acid synthesis. This higher level of FASN activity within cancer cells could be the reason why such cancer cells are able to sustain life.

In normal cells, barring liver and adipose tissue, the expression and activity of FASN is relatively low.[3] However, in the case of rapidly dividing cancer cells "fatty acids can be synthesized de novo in order to provide lipids for membrane formation and energy production via β-oxidation and lipid modification of proteins."[3] This leads to a high expression of FASN within these rapidly dividing cells, including prostate cancer, the leading cancer in terms of those affected. Thus, if FASN could be targeted and inhibited, perhaps the source of the vitally supplied fatty acids could be cut off and apoptosis would result.

This became the springboard for researchers Dr. Herman Odens and his colleagues.[4] They worked to create drugs that inhibited the thioesterase domain of fatty acid synthase. The structure of fatty acid synthase as well as the location of the thioesterase domain within the linear organization of the structure, can be seen below in **Figure 2.**

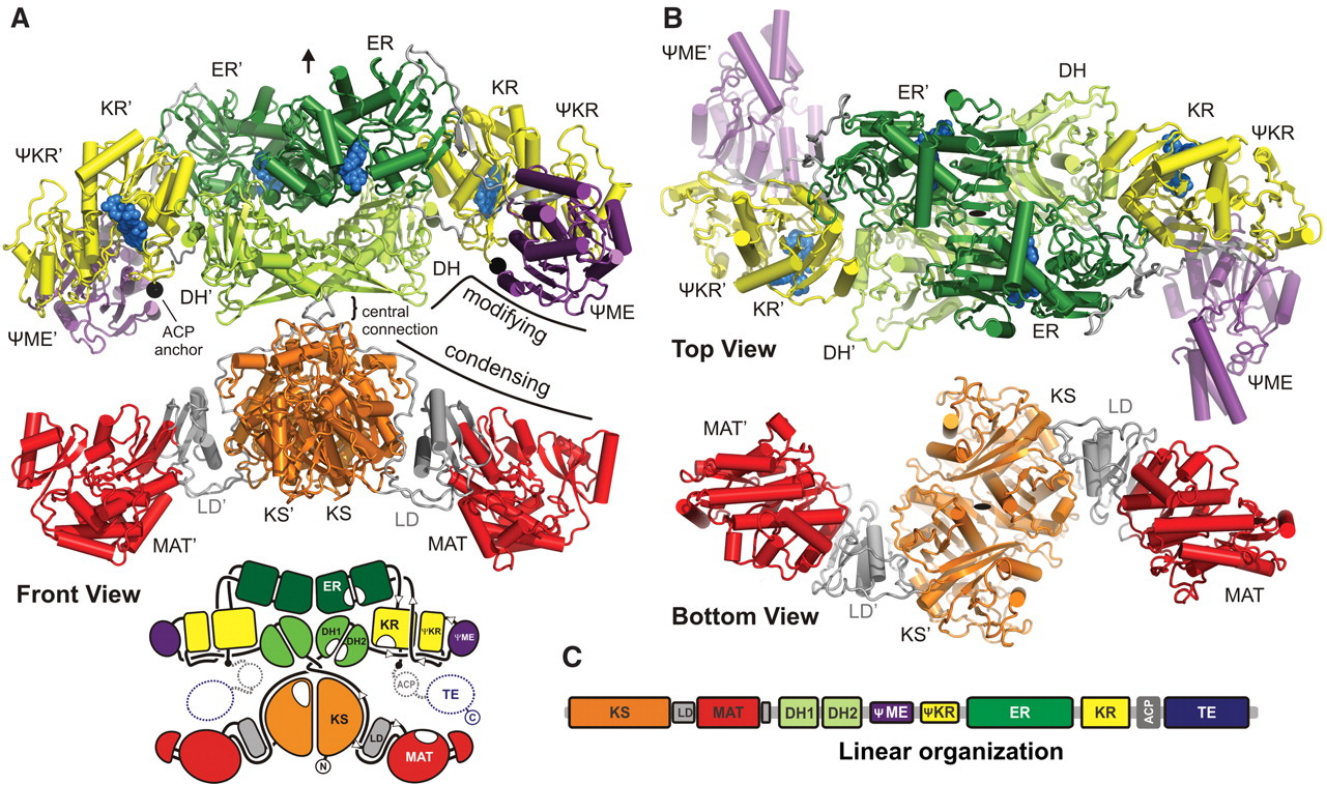

Figure 2. Crystal structure of a mammalian fatty acid synthase. Location of thioesterase domain within the linear organization of the structure seen in blue.[5]

Such drugs were found that successfully inhibited FASN by binding allosterically to the thioesterase domain of FASN. However, after toxicology studies were performed, these drugs were found to be too toxic for in vivo use. Research titled "Fatty acid synthase as a potential therapeutic target in cancer"[3] has suggested that the drugs Cerulenin, C75, Orlistat, C93 and

naturally occurring polyphenols have also been shown to inhibit FASN activity. These are seen in **Figure 3**.

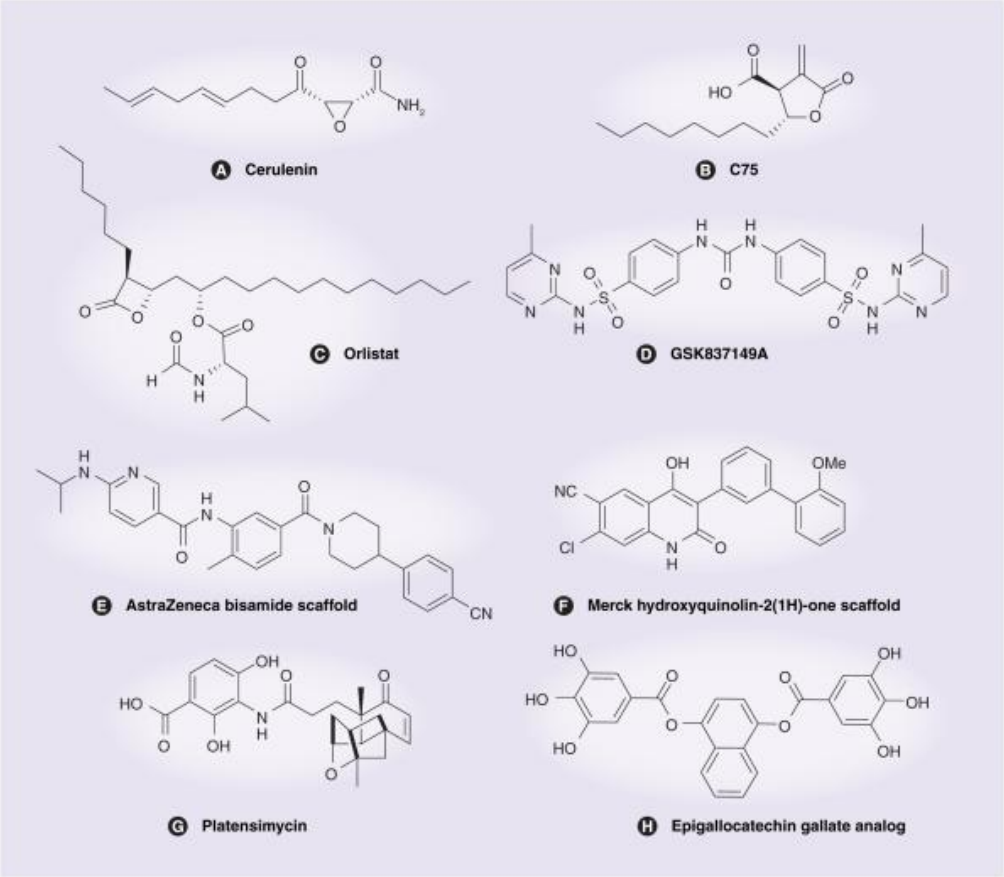

Figure 3. Drugs shown to inhibit FASN. [3]

In previous research, the procedure utilized in the making of this drug intermediate resembled closely the procedure employed by Dr. M S Rao and colleagues in their research.[6] This procedure used glacial acetic acid as a solvent system and involved harsh reaction conditions where the vial was left reacting overnight on a hotplate at 120^{o}C. The reaction was run using a one pot synthesis reaction and can be see in **Figure 4**. However, this method produced acetic acid impurity, and the harsh conditions were not ideal for synthesis.

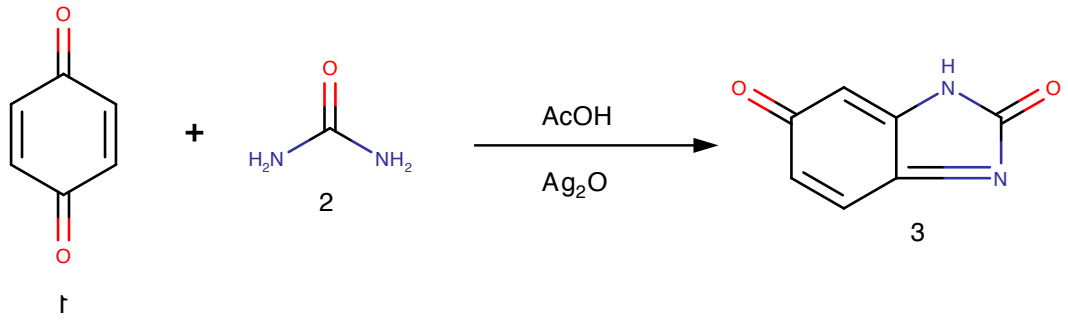

Figure 4. Synthesis of imidazole dione (3) using previous reaction method.

Therefore our goal for the research is to synthesize and design a new synthesis of 5-(alkylthio)-1H-benzo[d]imidazole-2,6-diones intended to successfully inhibit fatty acid synthase (FASN) by binding allosterically to the thioesterase domain of FASN, thus inhibiting the production of fatty acids in rapidly dividing cells. This will be accomplished through the synthetic scheme introduced in **Figure 5.**

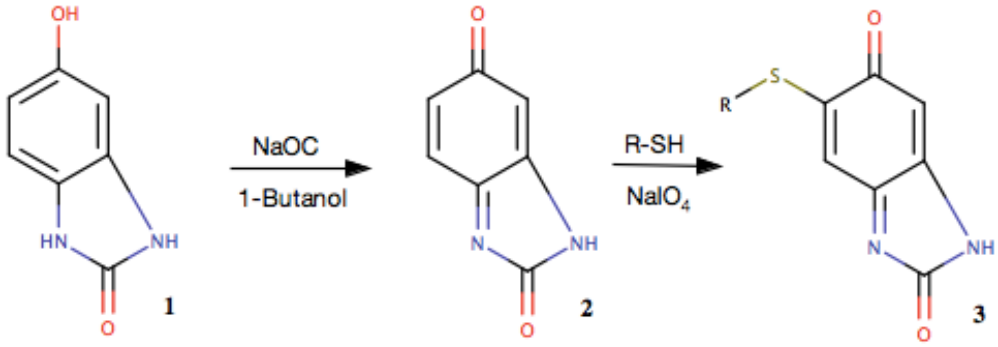

Figure 5. General reaction scheme for synthesis of new class of 5-(alkylthio)-1H-benzo[d]imidazole-2,6-diones.

The first step is the oxidation of the phenol which is a part of the six-membered ring, as well as the formation of a double bond between nitrogen and carbon of the five-membered ring. Next, a nucleophilic attack by the R-SH group adds an alkylthio to the ring, while the $NaIO_4$

serves as an oxidizing agent that reforms the oxidized product. The toxicity problem encountered by Dr. Odens and colleagues at Wake Forest was thought to have been caused by the the arrangement of the carbonyl groups on the original structure, which was found to successfully inhibit FASN. By rearranging the structure of the previous drug, we postulate the toxicity will be diminished.

Methods:

A small glass vial was used and six equivalents of sodium hypochlorite (NaOCl) was added to one equivalent of 5-hydroxy-1,3-dihydro-2H-benzimidazole-2-one along with 1 equivalent of 1-butanol. The reaction was covered using aluminum foil to avoid the breakdown of the NaOCl caused by exposure to light. A magnetic stir bar was placed in the vial and the reaction was stirred at 60^{o}C and left to react overnight. **Figure 6** is shown to illustrate the reaction mechanism described.

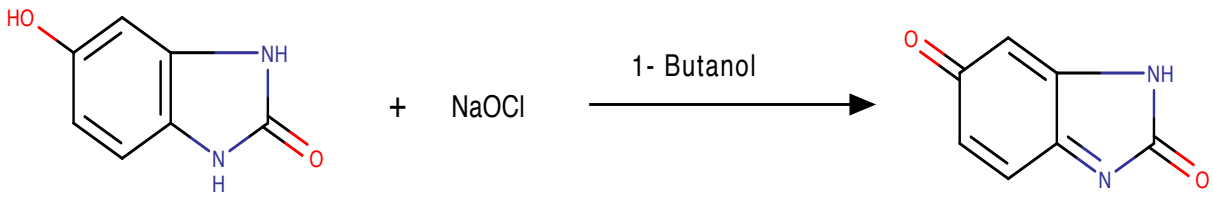

Figure 6. Oxidation of 5-hydroxy-1,3-dihydro-2H-benzimidazole-2-one to 1H-benzimidazole-2,6-dione.

The product was evaporated under reduced pressure using a Rotary Evaporator (Rotovap) and was then rinsed in a 9:1 DCM:MeOH solution and the extract liquid was evaporated again. A column was used to purify the liquid extract using a 95:5:0.01 DCM:MeOH:NH_3 solvent system and an ^{1}H-NMR was taken. Refer to results section.

Results:

In subsequent reactions the ratio of NaOCl to 5-hydroxy-1,3-dihydro-2H-benzimidazole-2-one was adjusted to create a stronger oxidizing effect. Originally we began with 2:1 equivalents NaOCl to starting material (5-hydroxy-1,3-dihydro-2H-benzimidazole-2-one) respectively. The reaction did not go to completion as was evidenced by the starting material being present on the TLC. Next we added four more equivalents leading to a 6:1 ratio of NaOCl to starting material and the reaction went to completion. This was confirmed with a TLC spot. We are currently experimenting to see if we can get a clean product using a 6:1 ratio. The current TLC and desired product that we are to isolating can be seen in **Figure 7.**

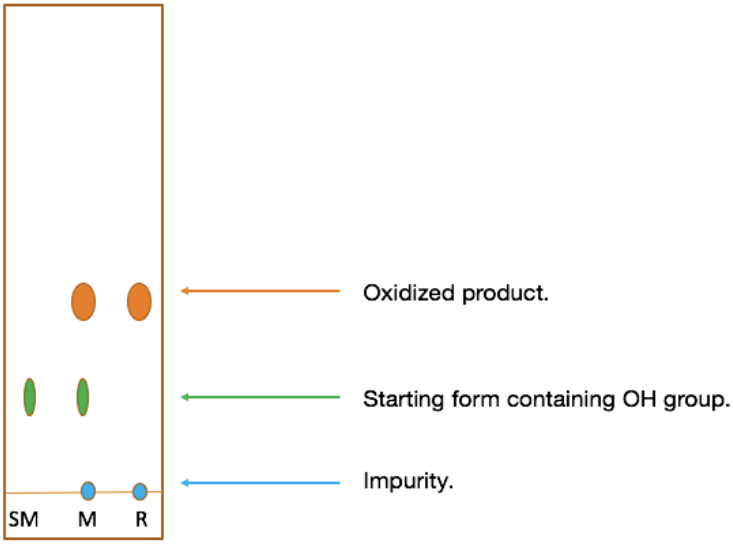

Figure 7. TLC spot from left to right the first column represents the starting material, the second is the mixture, and the far left column is the reaction. Green, orange, and blue spots represent starting form containing OH group, suspected oxidized product, and impurity respectively.

The ^{1}H-NMR data for the starting material used in the reaction seen in **Figure 6** is shown in **Figure 8**.

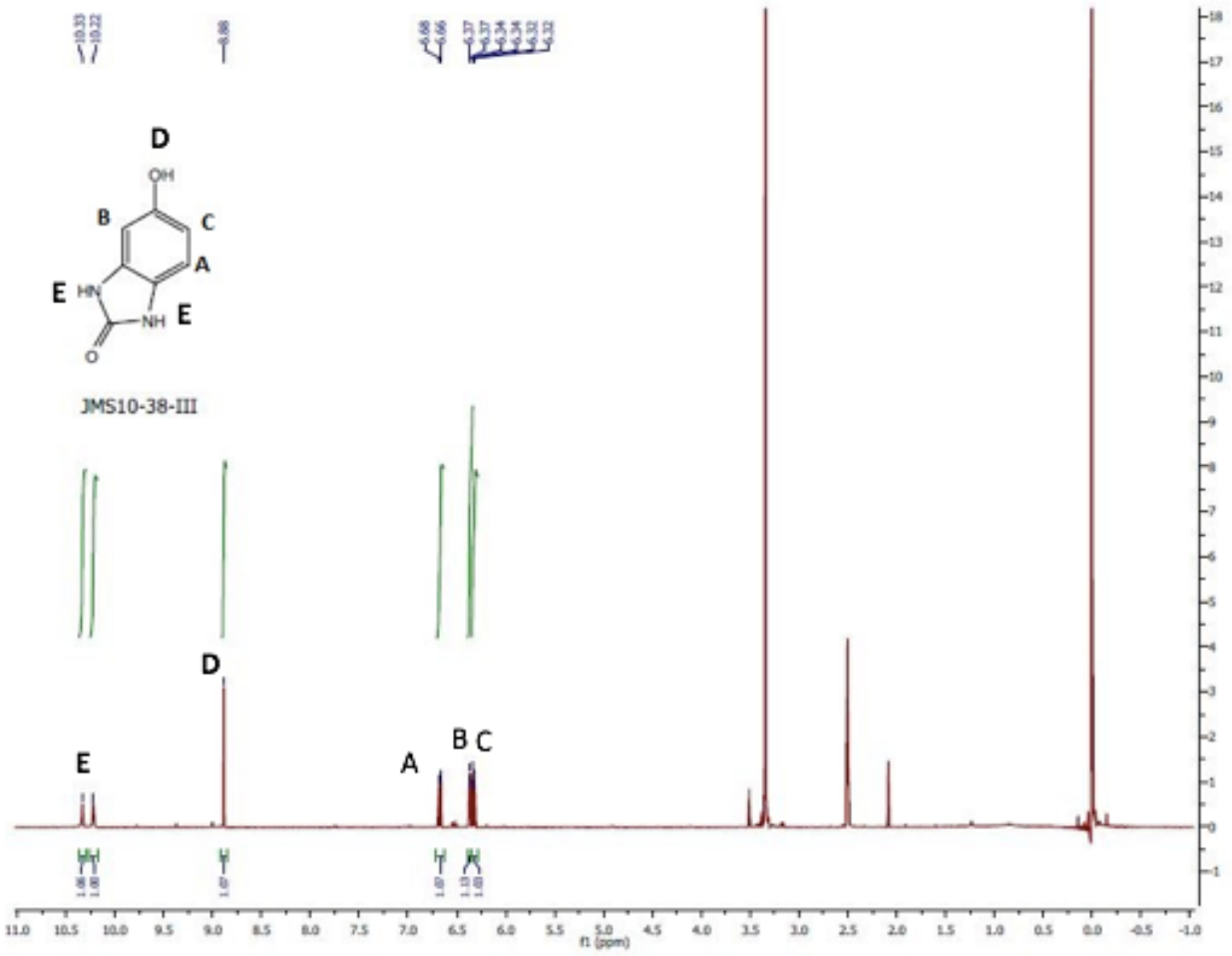

Figure 8. The ^{1}H-NMR shows the product formed by researcher Jon Sundin after purifying twice.

The ChemNMR ^{1}H predictor shown in **Figure 9** is used as a guide to show the theoretical and predicted positions of the hydrogen atoms. It can be used to match the ^{1}H-NMR for the sample to the structure.

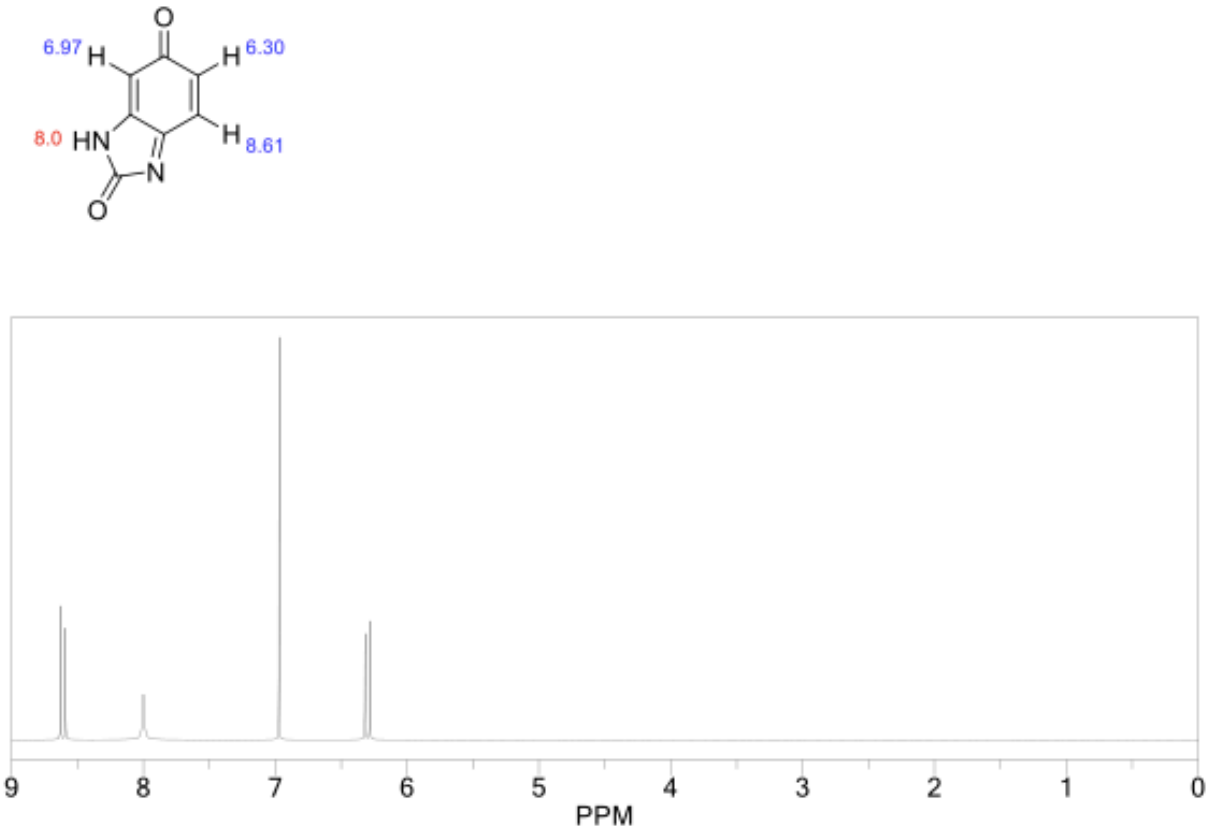

Figure 9. ChemNMR ^{1}H estimation shows the predicted structure and position of hydrogen atoms.

The first and second ^{1}H-NMR for the reaction shown in **Figure 6** is shown in **Figure 10** and **Figure 11** along with labeled hydrogens corresponding to the peaks.

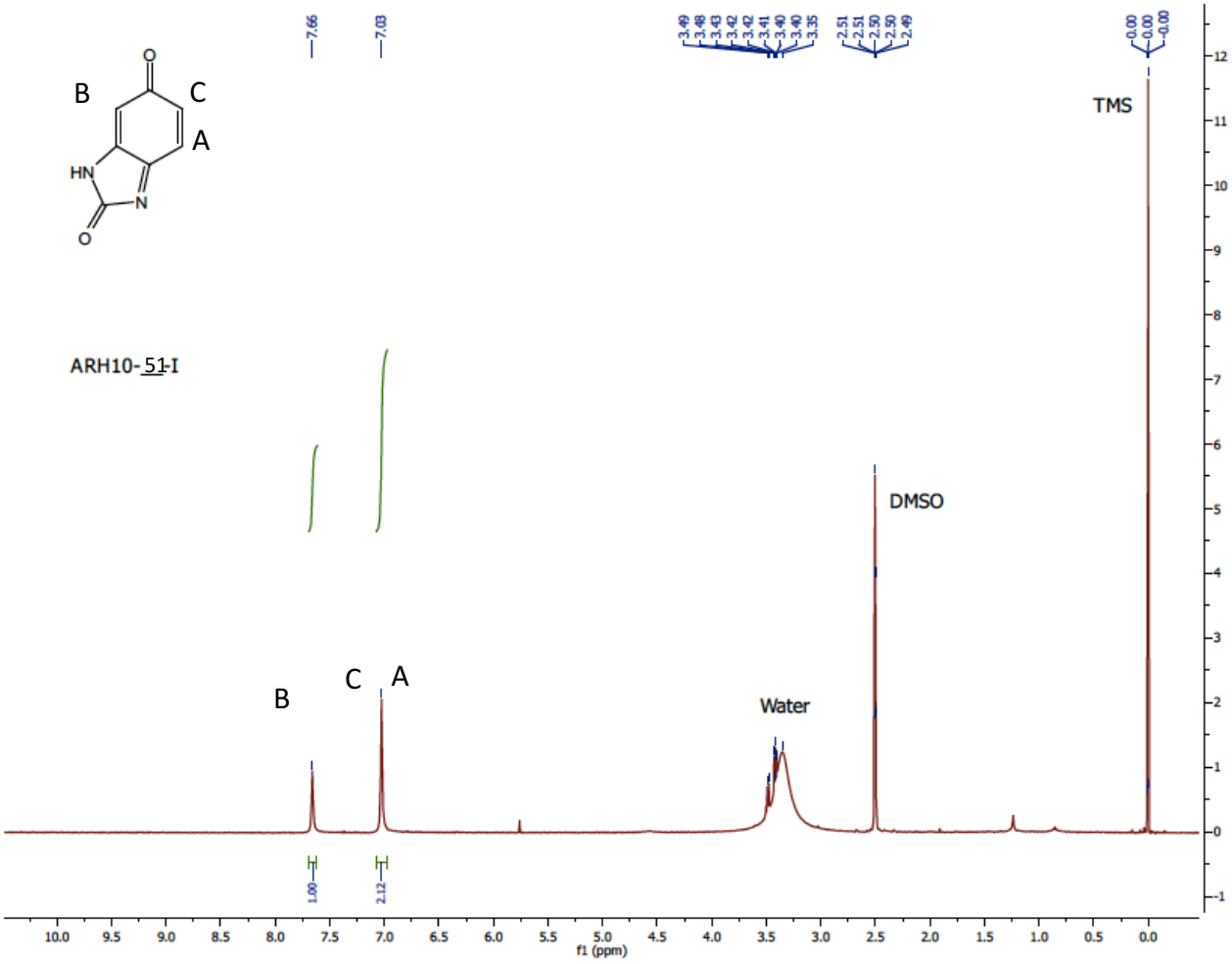

Figure 10. The [1]H-NMR shows the product formed after purification.

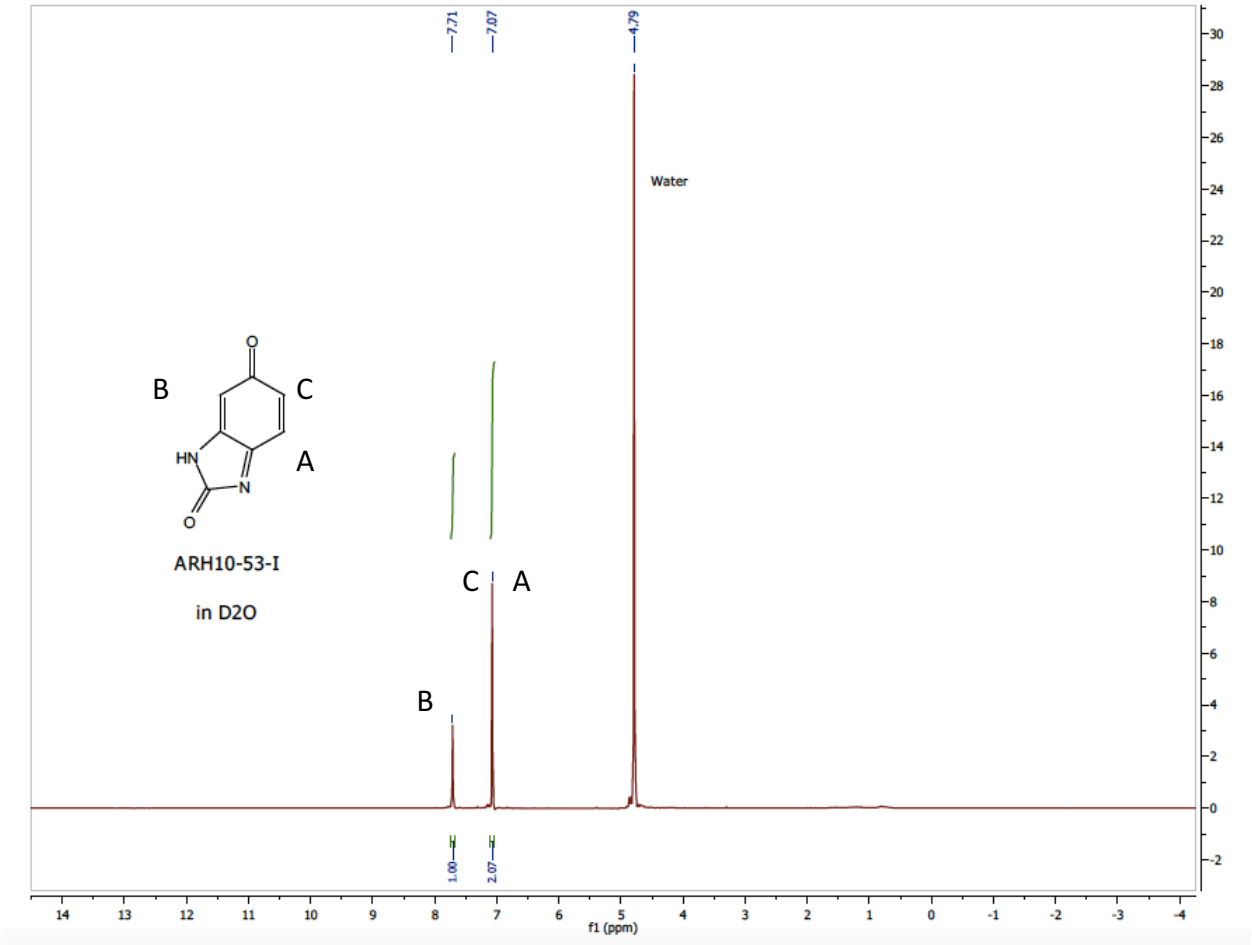

Figure 11. The ^{1}H-NMR shows the product formed after purification. This was the second time this product had been successfully synthesized.

Discussion:

After the initial reaction, we took a ^{1}H-NMR of our sample from the solid product we had obtained. This NMR did not show any conclusive results. Thus, the organic layer must be found in the liquid that was extracted from the solid. A purification of this extract was performed and the NMR results obtained after the purification are seen in **Figure 10** and **Figure 11**. The intended purpose of the purification is to isolate the orange spot seen on the TLC in **Figure 7**.

After further purifying our product, the ^{1}H-NMR showed positive results. Comparing **Figure 8** with **Figure 10**, the NMR data corresponding to the starting material and the oxidized product from the reaction, it is seen that the hydrogens labeled E and D do not show up in the NMR data of the product in **Figure 10** or **Figure 11.** This indicates that the product was successfully oxidized to the desired product according to reaction scheme seen in **Figure 6**. Therefore, the ^{1}H-NMR has confirmed the structure of the oxidized product.

Conclusion:

The focus of this step of the research was to carry out the oxidation of 5-hydroxy-1,3-dihydro-2H-benzimidazole-2-one to 1H-benzimidazole-2,6-dione through the use of sodium hypochlorite as an oxidizing agent as seen in **Figure 6**. This reaction was successfully carried out and and the product was purified. 1H-NMR can be used to confirm reaction product.[7,8] And this method was used to confirm the structure of the product. Now that the oxidation has successfully been performed, the next step is the addition of an alkylthio group to the six membered ring. This procedure will need to be explored, as ring substituents can often effect the compound's stability.[9] The procedure that is currently being proposed can be seen in **Figure 5.** Future work

could include comparing the toxicity of the current compound to other fatty acid synthase inhibitors.[10]

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